

Appl. No. 09/771,277
Amdt. dated January 6, 2004
Reply to Office Action of May 2, 2003

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS

Claim 1 (currently amended): A device for separating and detecting particles comprising:
a capillary having a first end and a second end, the capillary filled with a buffer solution,
the capillary having a coating that transforms the capillary into a light wave guide;

an electrical source for applying a voltage across the capillary, the voltage causing the
particles to travel from a first location within the capillary to a second location within the
capillary; and

an excitation source for rastering an excitation beam onto the capillary along part or all of
the length of the capillary, such that when a fluorescently labeled particle is positioned within the
capillary, the fluorescently labeled particle emits light after excitation with the excitation beam;
and

a light detector positioned at one of the first or second ends of the capillary to collect
fluorescent light emitted from the excited fluorescently labeled particles for determining the
location of particles within the capillary based upon the location of the rastered excitation beam;
~~the detector capable of determining the location of particles at more than one position along the
length of the capillary.~~

Claim 2 (original): The device of claim 1, further comprising a first reservoir in fluid
communication with the first end of the capillary, the first reservoir configured to contain buffer
solution and a second reservoir in fluid communication with the second end of the capillary, the
second reservoir configured to contain buffer solution

Claim 3 (canceled).

Appl. No. 09/771,277
Amdt. dated January 6, 2004
Reply to Office Action of May 2, 2003

Claim 4 (canceled).

Claim 5 (currently amended): The device of claim 1 ~~[[4]]~~, wherein the light detector further comprises a fiber optic coupled end-on to the capillary ~~a light detector positioned to collect fluorescent light emitted from the excited fluorescently labeled particles.~~

Claim 6 (original): The device of claim 5, wherein the light detector comprises low-level light detection electronics.

Claim 7 (previously amended): The device of claim 5, wherein the coating on the capillary that transforms the capillary into a light wave guide directs the fluorescent light toward the light detector.

Claim 8 (previously amended): The device of claim 1, wherein the coating has a refractive index number in the range from about 1.1 to about 1.4.

Claim 9 (previously amended): The device of claim 1, wherein the coating has a refractive index number of about 1.3.

Claim 10 (previously amended): The device of claim 8, wherein the coating is polytetrafluoroethylene.

Claim 11 (currently amended): The device of claim 1 ~~[[4]]~~, wherein the excitation beam has a power in the range from about 1 mW to about 1000 mW.

Claim 12 (currently amended): The device of claim 1 ~~[[4]]~~, wherein the excitation beam has a width in the range from about 5 μm to about 1000 μm .

Claim 13 (currently amended): The device of claim 1 [[4]], wherein the light detector can distinguish between more than one color of fluorescent light.

Claim 14 (currently amended): The device of claim 1 [[4]], wherein the light detector may be placed at either end or both ends of the separation capillary.

Claim 15 (currently amended): The device of claim 1, further comprising a plurality of capillaries.

Claim 16 (previously amended): The device of claim 1, wherein the buffer solution comprises a gel sieving material, a surface deactivating agent, and a buffer selected from the group consisting of tris-boric acid EDTA, potassium tartrate, and tris-acetate EDTA.

Claim 17 (original): The device of claim 16, wherein the gel sieving material is selected from the group consisting of poly(ethylene glycol), poly(vinyl alcohol), hydroxy propyl methyl cellulose, hydroxyethylcellulose, and linear polyacrylamide.

Claim 18 (original): The device of claim 16, wherein the surface deactivating agent is poly(vinylpyrrolidone).

Claim 19 (currently amended): A device for separating and detecting particles comprising:

- a capillary having a first end and a second end the capillary filled with a buffer solution, the capillary having a coating that transforms the capillary into a light wave guide;
- a first reservoir in fluid communication with the first end of the capillary, the first reservoir configured to contain buffer solution;

Appl. No. 09/771,277
Amdt. dated January 6, 2004
Reply to Office Action of May 2, 2003

a second reservoir in fluid communication with the second end of the capillary, the second reservoir configured to contain buffer solution;

an electrical source for applying a voltage across the capillary, the voltage causing a fluorescently labeled particle positioned within the capillary to travel from a first location within the capillary to a second location within the capillary;

an excitation source for directing an excitation beam onto the capillary, such that when a fluorescently labeled particle is positioned within the capillary, the fluorescently labeled particle emits light after excitation with the excitation beam;

the excitation source capable of exciting fluorescently labeled particles at more than one position along the capillary wherein the excitation beam is rastered along part or all of the length of the capillary; and

a light detector positioned to collect fluorescent light emitted from excited fluorescently labeled particle located within the capillary, wherein the light detector comprises a fiber optic coupled end-on to the capillary.

Claim 20 (previously amended): The device of claim 19, wherein the coating on the capillary that transforms the capillary into a light wave guide is capable of directing the fluorescent light toward the light detector.

Claim 21 (original): The device of claim 19, wherein the coating has a refractive index of about 1.3.

Claim 22 (original): The device of claim 19, wherein, the coating is polytetrafluoroethylene.

Claim 23 (canceled).

Claim 24 (original): The device of claim 19, wherein the light detector comprises low-level light detection electronics.

Claim 25 (original): The device of claim 24, wherein the low-level light detection electronics are selected from the group consisting of photomultipliers, photodiodes, and CCD cameras.

Claim 26 (original): The device of claim 24, wherein the light detector further comprises an optical filter, prism, grating, or light spectrometer positioned between the light detection electronics and the capillary for filtering incident light, and the resulting fluorescence.

Claim 27 (original): The device of claim 26, wherein the optical filter comprises a high band pass filter for filtering light with a wavelength greater than about 500 nm and a notch filter.

Claim 28 (original): The device of claim 26, wherein the optical filter comprises a narrow band pass filter which filters light other than light with a wavelength corresponding to the wavelength of the light emitted from the fluorescent label, ± 10 nm.

Claim 29 (canceled).

Claim 30 (original): The device of claim 19, wherein the excitation beam has a power in the range from about 1 mW to about 1000 mW.

Claim 31 (original): The device of claim 19, wherein the excitation beam has a width in the range from about 5 μm to about 1000 μm .

Appl. No. 09/771,277
Amdt. dated January 6, 2004
Reply to Office Action of May 2, 2003

Claim 32 (original): The device of claim 19, wherein the light detector can distinguish between more than one color of fluorescent light.

Claim 33 (original): The device of claim 19, further comprising a plurality capillaries.

Claim 34 (currently amended): The device of claim 19, wherein the buffer solution comprises a gel sieving material, a surface deactivating agent, and a buffer selected from the group consisting of tris-boric acid EDTA, potassium tartrate, and tris-acetate EDTA.

Claim 35 (original): The device of claim 34, wherein the gel sieving material is selected from the group consisting of poly(ethylene glycol), poly(vinyl alcohol), hydroxy propyl methyl cellulose, hydroxyethylcellulose, or linear polyacrylamide.

Claim 36 (previously amended): The device of claim 34, wherein the surface deactivating agent is poly(vinylpyrrolidone).

Claim 37 (original): The device of claim 34, wherein the gel sieving material is at a concentration in the range from about 0.1% to about 5% and has a viscosity in the range from about 0.5 cp to about 50 cp at room temperature.

Claim 38 (original): The device of claim 19, wherein the capillary has a length in the range from about 5 cm to about 100 cm.

Claim 39 (original): The device of claim 19, wherein the capillary has a length of about 20 cm.

Claim 40 (currently amended): A method for separation and sizing of particles in short channels by capillary electrophoresis comprising:

obtaining a sample of particles;

fluorescently labeling the particles;

loading the sample into a device for separating and sizing particles, the device comprising a capillary having a first end and a second end filled with a buffer solution, the capillary having a coating that transforms the capillary into a light wave guide, a first reservoir in fluid communication with the first end of the capillary, the first reservoir configured to contain the buffer solution, a second reservoir in fluid communication with the second end of the capillary, the second reservoir configured to contain the buffer solution, an electrical source for applying a voltage across the capillary, the voltage causing the fluorescently labeled particles to travel from a first location within the capillary to a second location within the capillary, an excitation source for directing an excitation beam onto fluorescently labeled particles within the capillary, the fluorescently labeled particles emitting fluorescent light after excitation with the excitation beam, the excitation source capable of exciting the fluorescently labeled DNA particles at more than one position along length of the capillary, and a light detector positioned to collect the fluorescent light emitted the excited fluorescently labeled particle;

applying the voltage across the capillary;

rastering the excitation beam on the capillary;

monitoring fluorescent light in the light detector; and

comparing the position of the excitation beam on the capillary when light is collected by the light detector to determine the position of the particles in the capillary; and

determining the relative size of the particles from the determined position.

Claim 41 (previously amended): The method of claim 40, wherein the device further comprises at least one additional capillary having a first end and a second end, the at least one additional capillary filled with buffer solution and having a coating that transforms the additional

capillary into a light wave guide, the at least one additional capillary being in fluid communication with the first and second reservoirs, the method further comprising obtaining a second sample of particles of a known size, fluorescently labeling the particles of the second sample, applying the voltage across the at least one additional capillary, rastering the excitation beam on the at least one additional capillary, monitoring the collection of fluorescent light in the light detector; and comparing the position of the excitation beam on the capillary when light is collected by the light detector to determine the position of the particles of known size, comparing the position of the particles of known size to the position of the sample particles to determine the size of the sample particles.

Claim 42 (original): The method of claim 41, wherein the voltage is in the range of about 4,000 V to about 20,000 V dc.

Claim 43 (original): The method of claim 41, wherein the capillary has a length in the range of about 5 cm to about 100 cm.

Claim 44 (original): The method of claim 43, wherein the length is in the range of about 10 cm to about 25 cm.

Claim 45 (original): The method of claim 43, wherein the length is in the range of about 20 cm.

Claim 46 (original): The method of claim 41, wherein the particle is selected from the group consisting of a nucleic acid, a protein, inorganic ions, and organic ions, and neutral species.

Claim 47 (previously amended): The method of claim 41, wherein the coating on each of the capillaries that transforms the capillaries into a light wave guide directs the fluorescent light toward the light detector.

Claim 48 (previously amended): The method of claim 41, wherein the coating has a refractive index of about 1.3.

Claim 49 (previously amended): The method of claim 41, wherein the coating comprises polytetrafluoroethylene.

Claim 50 (original): A method for sequencing DNA comprising:
obtaining a sample of DNA to be sequenced;
running a dideoxy sequencing reaction on the DNA sample, the sequencing reaction comprising a separate reaction mixture for each nucleotide type, each reaction mixture comprising a different fluorescent label, each reaction mixture run to form a separate reaction product;
pooling the reaction products of the reaction mixtures;
loading the pooled reaction products into a device for separating and detecting particles, the device comprising a capillary having a first end and a second end filled with a buffer solution, a first reservoir in fluid communication with the first end of the capillary, the first reservoir configured to contain the buffer solution, a second reservoir in fluid communication with the second end of the capillary, the second reservoir configured to contain the buffer solution, an electrical source for applying a voltage across the capillary, the voltage causing the fluorescently labeled reaction products to travel from a first location within the capillary to a second location within the capillary, an excitation source for directing an excitation beam onto the fluorescently labeled reaction products within the capillary, the fluorescently labeled reaction products emitting fluorescent light after excitation with the excitation beam, the excitation source

Appl. No. 09/771,277
Amdt. dated January 6, 2004
Reply to Office Action of May 2, 2003

capable of exciting the fluorescently reaction products at more than one position along length of the capillary, and a light detector positioned to collect the fluorescent light emitted the excited fluorescently labeled reaction products;

- applying the voltage across the capillary;

- rastering the excitation beam on the capillary;

- monitoring the collection of fluorescent light in the light detector; and

- comparing the position of the excitation beam on the capillary to the color of light detected by the light detector to determine the position of a corresponding nucleotide within the DNA sample.